California Institute for Regenerative Medicine

Dissecting the Roles of miR-302/367 Cluster in Cellular Reprogramming Using TALE-based Repressor and TALEN.

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Public Summary:
MicroRNAs are important gene regulators involved in many biological processes, including stemness maintenance and cellular reprogramming. Due to their unique gene structures and small sizes, there is no efficient or simple strategy to knock down or knock out microRNAs or whole microRNA clusters, in order to investigate their effects on gene regulation. Here, we demonstrate knockdown of the miR-302/367 cluster by using the Kruppel-associated box repressor domain fused with specific transcription activator-like effectors (TALEs) designed to bind the miR-302/367 cluster promoter. We also designed two pairs of TALE nucleases (TALENs) to efficiently delete the miR-302/367 cluster in primary human fibroblasts and determined that knockout of the miR-302/367 cluster completely blocked induced pluripotent stem cell (iPSC) generation. Together, our results demonstrate that TALE-based transcriptional repressor and TALENs are two promising approaches for loss-of-function studies of microRNA clusters in somatic cells and pluripotent stem cells.

Scientific Abstract:
MicroRNAs are important gene regulators involved in many biological processes, including stemness maintenance and cellular reprogramming. Current methods used in loss-of-function studies of microRNAs mainly include locked nucleic acid (LNA) oligonucleotides and miRZip inhibitors, which have several limitations. Due to their unique gene structures and small sizes, there is no efficient or simple strategy to knock down or knock out microRNAs or whole microRNA clusters. Here, we demonstrate knockdown of the miR-302/367 cluster by using the Kruppel-associated box repressor domain fused with specific transcription activator-like effectors (TALEs) designed to bind the miR-302/367 cluster promoter. We also designed two pairs of TALE nucleases (TALENs) to efficiently delete the miR-302/367 cluster in primary human fibroblasts and determined that knockout of the miR-302/367 cluster completely blocked induced pluripotent stem cell (iPSC) generation. Together, our results demonstrate that TALE-based transcriptional repressor and TALENs are two promising approaches for loss-of-function studies of microRNA clusters in somatic cells and pluripotent stem cells.

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